Remarks

Claims 1-43 are pending in the subject application. By this Amendment, Applicants have amended claims 1, 3, 4, 6, 7-10, 13, 16-19, 23, 27, 31, 32, 34, 35-39, and 41 and added new claims 44-50. Support for the new claims and amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 1-50 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

As an initial matter, Applicants note that the specification has been amended at page 17, line 1, to correct a grammatical error (an incomplete sentence). Entry of the amendment is respectfully requested. Accordingly, reconsideration and withdrawal of this objection is respectfully requested.

Claims 16 and 17 are objected to because of informalities. The Examiner indicates that the phrase "incubating the sample for at room temperature" in claim 16 is nonsensical. Applicants gratefully acknowledge the Examiner's careful review of the claims. By this Amendment, Applicants have deleted the word "for" in claim 16. Accordingly, reconsideration and withdrawal of the objection is respectfully requested.

Claims 16-43 are rejected under 35 USC §112, second paragraph, as indefinite. Claims 16 and 17 are rejected on the grounds that the limitation "the dried reagent(s)" lacks antecedent basis. Applicants have amended claim 16 to replace "dried reagent(s)" with "plasminogen and streptokinase." Claims 16-19 are also rejected on the grounds that the limitation "the solution" lacks antecedent basis. In addition, claim 16 has been amended to replace the phrase "the solution" with the phrase "the buffer solution." Applicants respectfully assert that the claim language finds antecedent basis in the claims. Applicants have also amended claims to replace trademark names with generic names and to add further clarity to the subject matter being claimed. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §112, second paragraph, is respectfully requested.

Claims 1, 7, and 8 are rejected under 35 USC §102(b) as anticipated by Zhang *et al.* (1995). The Examiner asserts that Zhang *et al.* (1995) teach a method of collecting blood samples from patients and the blood is then broken up with buffer ATL which comprises SDS and Proteinase K.

The purified DNA is then subjected to PCR amplification, wherein the presence of bacterial DNA in patient's blood is detected. Applicants respectfully traverse this ground of rejection.

Applicants respectfully assert that the Zhang *et al.* reference does not teach or suggest the claimed invention. The Zhang *et al.* reference teaches pre-treatment of the blood by "vortexing" the blood with glass beads for 5 minutes. The vortexing of blood breaks up the blood sample itself so that it may be efficiently broken down by proteinase K. In contrast, the claimed invention does not require the breakdown of the blood matrix using glass beads as a pre-treatment to process blood nor the combination of this treatment with the reagents cited by Zhang *et al.* and used widely for small blood samples. Applicants' claimed methods utilize enzymes, (*e.g.,* DNase, plasminogen, streptokinase, phospholipase, endonuclease, lipase), not vortexing with glass beads, to pre-treat blood.

In addition, the Zhang *et al.* methodology is suitable only for small sample sizes, *e.g.,* 0.5 ml whole blood sample inputs. Small sample sizes are relatively easy to process using the method taught by Zhang *et al.* as the level of contaminants, such as PCR inhibitors, that are widely known to sometimes contaminate blood sample nucleic acid extracts, will be sufficiently low in the final extract. The subject invention teaches a method for processing large volume whole blood used to detect low levels of bacterial particles (10 – 40 CFU / 6 ml). The use of (in one embodiment) 12 times (6 ml) the amount of blood used by Zhang *et al.* (0.5 ml) is known to contaminate purified nucleic acid extracts with human DNA, heme, and other iron species. These contaminants are well known in the art to be inhibitory for PCR methodology.

When 6 ml blood samples are treated with detergent and centrifuged, the sediment is very abundant and very difficult to digest with proteinase K reagents such as described by Zhang *et al.* Such samples often result in contaminated and inhibitory nucleic acid extracts. In contrast, when the methods of the subject invention, including combinations of phospholipase A_2 and DNase, are used in combination to treat 6 ml samples the blood matrix is successfully broken down so that sediment is easily processed by proteinase K digestion (see, for example, Figure 18 of the subject specification).

In order to anticipate, a single reference must disclose within the four corners of the document each and every element and limitation contained in the rejected claim. *Scripps Clinic & Research Foundation v. Genentech Inc.*, 18 USPQ2d 1001, 1010 (Fed. Cir. 1991).

Accordingly, reconsideration and withdrawal of the rejection under 35 USC §102(b) is respectfully requested.

Claims 1 and 2 are rejected under 35 USC §102(b) as anticipated by Cassels *et al.* (1987). In addition, claims 1-6 are rejected under 35 USC §103(a) as obvious over Cassels *et al.* (1987) in view of Dupe *et al.* (1981). The Cassels *et al.* (1987) reference is cited as teaching a method of adding plasminogen/streptokinase combination into an assay medium containing Tween 80, a detergent, and that the efficacy of the enzyme combination is tested by measuring ^{125}I remaining in the plasma which is a measure of how much fibrin was cleaved by the streptokinase/fibrinogen combination. Additionally the Cassels *et al.* reference is cited as teaching that streptokinase assays can be carried out in a buffer comprising potassium phosphate. The Examiner asserts that Dupe *et al.* (1981) teaches that it is possible to freeze plasminogen which has been activated by streptokinase in a buffer comprising sodium chloride. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Cassels *et al.* teach an enzymatic reaction in a 0.9 ml plasma sample for the purpose of measuring endogenous enzyme activity. In contrast, the claimed invention can be used for analyzing large volume (6.0 ml) whole blood samples for the presence of bacteria that have occurred as part of a pathogen infective state. Plasma is known in the art to be the easiest to process clinical samples and whole blood is considered along with feces to be the most difficult clinical samples. Blood is considered one of the most difficult of all samples to process.

In addition, the Cassels *et al.* reference is focused on testing for properties of the blood itself while the subject invention is directed to methods for treating whole blood in order to be able to detect the presence of particles such as bacteria and virus in the blood sample.

The addition of the streptokinase and plasminogen plus detergent facilitates the filtration of large quantity whole blood samples through 0.2 μm Polyethersulfone filters as demonstrated by the

subject invention for bacterial nucleic acid purification (see paragraphs 0049 and 0061 of the subject specification).

The use of the streptokinase and plasminogen plus detergent in the claimed method facilitates movement of the subject invention treated whole blood sample through microfluidic devices and facilitates analyte capture signature identification by virtue of making the blood less viscous and molecular recognition sites to be more consistently able to bind (see paragraphs 0062 and 0063).

When 6 ml blood samples are treated with the subject invention of plasminogen freshly activated by streptokinase, the appearance on the filter surface of tissue aggregates formed from fibrin and DNA can be used to measure the extent of molecular breakdown for the blood sample (see Figure 19). Such aggregates can be seen on microscopy as 0.5 – 5.0 μm long twisted strands and are eliminated by treatment with DNase. An increase in plasminogen can reduce the need for DNase to achieve the same level of sample breakdown. The combination of plasminogen freshly activated by streptokinase and DNase to prevent the formation of tissue aggregates is not anticipated by the work of Cassels *et al.* where there is no challenge to prevent fibrin DNA aggregates from forming.

The Cassels *et al.* reference is also cited as teaching use of potassium phosphate as a true buffer in a streptokinase assay. The subject invention pertains to potassium phosphate as a component for a complex enzyme cocktail which also contains DNase and Phospholipase A_2. The potassium phosphate is used in combination with other elements described herein. The potassium phosphate is not used as a true buffer where the method is conducted at pH 7.8 to 8.0 (optimal for Phospholipase A_2 activity). The subject invention uses magnesium as the divalent cation source for catalyzing DNase reactions. Magnesium is known in the art to react and produce a precipitant when combined with potassium phosphate in solution. The subject invention teaches against the art in how potassium phosphate is used in a method where insoluble elements are produced and where the subject method is practiced at a pH outside the useful range for potassium phosphate. The subject method is practiced to drive molecularly destructive processes as the primary objective and not to pursue the result of the process in order to biochemically define the endogenous components of a plasma sample. Thus, the Cassels *et al.* reference does not teach or suggest the claimed invention.

The Dupe *et al.* reference describes activation of plasminogen with streptokinase and subsequent storage of the mixture at – 20 degrees in an unremarkable Tris buffered NaCl glycerol

storage buffer known in the art for preserving enzyme activity at all temperatures. The Dupe *et al.* reference, like Cassels *et al.*, also describes the testing of plasma samples for endogenous elements. The work by Dupe *et al.* is not biochemically appropriate to compare to the subject invention in that a different sample (plasma verses whole blood) and objective (defining plasma components verses concentrating and extracting particles (*e.g.*, bacteria)) are targeted. The methodology described by Dupe *et al.* in fact does not work for the subject invention application.

In addition, Applicants have conducted experimental work wherein the use of a frozen preactivated plasminogen mixture failed to function in whole blood samples. The frozen preactivated plasminogen mixture prepared in a NaCl glycerol storage buffer completely failed to enzymatically react with fibrin in a whole blood sample. Applicants note that Streptokinase preactivated plasminogen rapidly declines to a total loss in activity (as observed in the purified nucleic acid product resulting from combining with proteinase K) within 5 minutes from activation and storage on ice in Tris buffered NaCl glycerol storage buffer as described by Cassels *et al.*

The subject invention (reacting streptokinase and plasminogen just before or when whole blood is exposed to the reagents) succeeds while the Dupe *et al.* method of pre-activation (reacting streptokinase and plasminogen and storage on ice or in a frozen state) fails in the detection of particles in whole blood samples.

As the Examiner is aware, it is well established in patent law that in order to support a *prima facie* case of obviousness, a person of ordinary skill in the art must find both the suggestion of the claimed invention, and a reasonable expectation of success in making that invention, solely in light of the teachings of the prior art. *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). Accordingly, reconsideration and withdrawal of the rejections under 35 USC §102(b) and 35 USC §103(a) is respectfully requested.

Claims 1 and 7-9 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Heininger *et al.* (2001). Claims 1, 7, 8, and 10 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Garg *et al.* (1996). The Zhang *et al.* reference is cited for its teachings as before. The Examiner asserts that Heininger *et al.* (2001) teaches the addition of recombinant DNAase to blood samples that are also inoculated with bacterial DNA and the use of PCR to identify bacterial DNA in serum. The Examiner asserts that the Garg *et al.* (1996) reference

teaches a method of isolating DNA from white blood cells in whole blood and that their method teaches that the lysate can be incubated with RNAse to remove RNA and that PCR can successfully be performed on the DNA isolated from the RNAse-treated blood lysate. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.* reference. The secondary references cited under this rejection do not cure the deficiencies of the primary reference.

The Heininger *et al.* reference is cited as teaching that one can add DNase to serum samples that also contain bacterial DNA and that adding DNase to blood does not significantly inhibit its usefulness in PCR assays to detect bacterial DNA. As noted previously herein, plasma and serum are known in the art to be among the easiest to process clinical samples while whole blood is considered to be one of the most difficult clinical samples to process. The addition of high levels of DNase (with out the aurintricarboxylic acid embodiment described herein) to blood samples significantly inhibits the samples usefulness in PCR. Applicants have taught that by combining DNase with streptokinase freshly activated plasminogen and/or phospholipase can one derive purified nucleic acid extracts that allow detection of 10 – 40 CFU bacterial particles found in 6 ml whole blood. Accordingly, Applicants respectfully assert that the Heininger *et al.* reference is irrelevant in that the methods described therein will not function as a sensitive clinical diagnostic tool.

Applicants also note that Heininger *et al.* used serum samples seeded with 1,000 times higher levels of target bacterial particle DNA than what is targeted by the subject invention. Targeting the detection of low level pathogen provides clinical relevance.

Heininger *et al.* test at levels of bacterial DNA far too high to be clinically useful or reasonably compared to an ultra sensitive pathogen diagnostic method where the challenge to detect is much more pronounced. Detecting 10 – 40 CFU bacterial particles targets with levels of DNA measured not by grams but by numbers of molecules (copies) exponentially more difficult when 6 ml whole blood is the starting sample input.

Heininger *et al.* sought to prove that DNase can be present with *Escherichia coli* naked bacterial DNA in serum samples used for PCR testing. The objective for using DNase as described in the subject invention is to minimize the size of fibrin DNA aggregates that form when whole blood samples are lysed with detergents and or enzymes. By minimizing the size of the fibrin aggregates proteinase K can act upon more active sites in order to ensure a functional nucleic acid extract for PCR is produced when starting with 6 ml blood samples. The aggregates can be seen via microscopy when detergent is used to treat blood that is then centrifuged and the pellet is prepared with PAP differential staining reagents known in the art. When DNase is omitted from methods to analyze and filter whole human blood, the fibrin aggregates are observed in the filter surface. The subject invention provides a method to break down human DNA while preserving bacterial DNA found in very low levels of pathogen.

Heininger *et al.* describe levels of DNase that would completely eliminate PCR detection of many biological threat agents and common clinical pathogens such as *Pseudomonas aeruginosa* seeded at 1,000 CFU or less per 6 ml whole blood sample. Use of the levels of DNase described by Heininger *et al.* would destroy PCR detection of bacterial pathogens at levels such pathogens are expected to occur when patients present with symptoms where a maximal impact on quality of care is greatest. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, 12, 16, 17, and 23 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987) and Smith *et al.* (1982). The Zhang *et al.* and Cassels *et al.* references are cited for their teachings as before. Cassels *et al.* is claimed to demonstrate use of streptokinase activation of plasminogen at ambient temperature. The Examiner asserts that the Smith *et al.* (1982) reference teaches that one can mix streptokinase with plasminogen and freeze dry it into vials and that one can reconstitute it and use it to lyse fragments from fibrin clots *in vivo* in rabbits. The Examiner also asserts that Smith *et al.* teach that it is desirable to reconstitute freeze-dried enzymes in a solution before addition to blood. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do <u>not</u> teach or suggest Applicants' claimed invention. Applicants respectfully reassert

and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.* and the Cassels *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references.

Applicants respectfully assert that resuspension of streptokinase activated plasminogen in freeze drying reagents present the same rapid (within 5 minutes) loss of processivity in the whole blood sample used for pathogen nucleic acid extraction. The method of preserving activity for streptokinase activated plasminogen via freeze drying as described by Smith is irrelevant to the subject invention since activation of plasminogen by streptokinase at any time other than just before exposure to the whole blood sample produces contaminated nucleic acid extracts that are inhibitory to PCR. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, and 13 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), Garg *et al.* (1996), Heininger *et al.* (2001), Benjamin *et al.* (1998), and Grotendorst *et al.* (1999). The Zhang *et al.*, Cassels *et al.*, Smith *et al.*, Garg *et al.*, and Heininger *et al.* references are cited for their teachings as before. The Examiner asserts that the Benjamin *et al.* (1998) reference teaches that lipase is useful in making clinical diagnosis from blood because lipases hydrolyze lipids in blood samples creating measurable metabolites that allow for diagnosis of medical conditions. The Examiner asserts that the Grotendorst *et al.* (1997) reference teaches that phospholipase A_2 has hemolytic properties, and thus is useful for removing cells from blood. Applicants respectfully traverse these grounds of rejection. Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.*, Cassels *et al.*, Smith *et al.*, Garg *et al.*, and Heininger *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references.

Benjamin *et al.* teach measuring the products of enzyme catalysis for endogenous blood elements as a diagnostic tool. The subject invention employs phospholipase A_2 to digest the endoplasmic reticulum and DNase to digest blood cell DNA into short fragments. Benjamin *et al.*

and Gortendorst *et al.* do not include the detergents saponin and/or methyl 6-O-(N-heptylcarbamoyl)-α-D-glucopyranoside.

Grotendorst *et al.* describe how phospholipase A_2 dissolve cell membranes. The subject invention is not directed to the use of phospholipase A_2 to dissolve blood components for directly facilitating the isolation of bacterial particles. Phospholipase A_2 is included in one embodiment of the subject invention, in combination with DNase, to breakdown the endoplasmic reticulum in order to allow DNase better access to the blood cell DNA. By properly breaking down the blood cell DNA, the sample can be more efficiently digested with proteinase K and the blood cell DNA contributes a less inhibitory element to PCR testing. By opening the nucleolus with phospholipase A_2, short fragments of blood cell DNA result from digestion with DNase. The short fragments are not quantitatively carried through existing nucleic acid extraction technologies and hence are not able to inhibit PCR.

When the subject invention is practiced where post enzyme treatment and centrifugation sediment quantity is measured, as is reported in Figure 18 of the subject specification, the ordinarily skilled artisan can understand that Phospholipase A_2 or DNase utilized separately do not produce a diminished biomass in the sediment. The smallest sedimentary pellet (denoting maximum molecular breakdown of blood elements) was produced when DNase and phospholipase A_2 were used in combination. This pellet went into solution the fastest when using a typical proteinase K digestion reagent.

Applicants also note that the nonobviousness of the claimed invention is evidenced by the fact that no reference taught or suggested using a combination of phospholipase A_2 and DNase to isolate low level bacterial particles despite abundant efforts and financial resources to accomplish such an objective. A large amount of funding has been provided by the U.S. Government since 2001 to the National Institutes for Allergy and Infectious Disease in part to support the development of rapid detection for clinical involvement of biological threat agents in support of protecting the populace. Despite funds being available for competitive support, no entity was funded to develop a detection system that included blood sample preparation methods that would produce clinically significant and representative samples which allow detection of biological threat agents at low levels (10 – 40 CFU /6-10 ml blood) when intervention is more likely to be beneficial. It is this matter of

clinical significance that the subject invention addresses by concentrating and purifying large volume (6 – 10 ml) whole blood into a small volume (0.1 – 0.2 ml) of purified nucleic acid extract.

The subject invention solves a problem for processing large volume blood samples into purified nucleic acid extract in support of detecting 10 – 40 CFU of bacteria per 10 ml of whole blood. The subject invention has been evaluated in collaboration with numerous Academic, Corporate, Military, and Public Health entities to explore pathogen detection ideas.

It has been clear that no technology other than the subject methods can process blood for the low level detection capability described above. Applicants respectfully assert that it is an indicia of nonobviousness of the claimed methods using phospholipase A_2 in combination with DNase to process blood for low level pathogen PCR detection in that it has it not been taught or described by some of the largest pathogen detection technology developers in the world.

Global corporate entities project the U.S. market for clinical pathogen nucleic acid diagnostics to be worth one hundred million dollars. Despite spending sixteen billion dollars in the United States each year on treating sepsis, over two hundred thousand lives are lost domestically and this number is expected to grow. Despite the existence of technologies to detect bacterial particles and a large financial incentives to develop and market such products the solution provided by the subject invention to use phospholipase A_2 combined with DNase has not been described by others.

The modern clinical diagnostics market for blood borne pathogens is beset with tremendous difficulties which impede medical relevancy due to too small a blood sample also being non representative and inhibitory. Currently, throughout the world, slow blood culture pathogen detection methods are found to be useless, are often not used for emergency department admissions, and primarily benefit the next patients in epidemiological terms. Recent findings from the anthrax attacks of 2001 suggest rapid highly sensitive diagnostics would have been useful. Thus, there remains a clear need in the art for the development of highly sensitive pathogen detective methods.

Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, and 13-15 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), Garg *et al.* (1996), Heininger *et al.* (2001), Benjamin *et al.* (1998), Grotendorst *et al.* (1999), and Krielgaard *et al.* (1998). The Zhang *et*

al., Cassels *et al.*, Smith *et al.*, Garg *et al.*, Heininger *et al.*, Benjamin *et al.*, and Grotendorst *et al.* references are cited for their teachings as before. The Examiner asserts that the Kreilgaard *et al.* (1998) reference teaches that the addition of trehalose to a protein before the freeze drying process affords protection to enzymes during freeze drying and storage as a dried solid. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.*, Cassels *et al.*, Smith *et al.*, Garg *et al.*, Heininger *et al.*, Benjamin *et al.*, and Grotendorst *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, 16, and 18-22 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), and Diez *et al.* (1999), and further in view of Hallick *et al.* (1977) and Von Pape *et al.* (2000). The Zhang *et al.*, Cassels *et al.*, and Smith *et al.* references are cited for their teachings as before. The Examiner asserts that the Diez *et al.* (1999) reference teaches that one can recover mostly intact RNA by treating cells with DEPC, a chemical that inhibits RNAse and that this is effective in producing a sample that can be used with PCR for generating clean signal. The Examiner asserts that the Hallick *et al.* (1977) reference teaches that aurintricarboxylic acid (ATA) is a general nuclease inhibitor, the addition of ATA to a nuclease reaction inhibits the reaction, and that it would be useful to add ATA to prevent degradation of nucleic acids during nucleic acid isolation. The Examiner asserts that the Von Pape *et al.* (2000) reference teaches that addition of sodium citrate has anticoagulant properties and that its addition to a blood sample has anticoagulant properties. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.*, Cassels *et al.*, and Smith *et al.* references. The secondary references

cited under this rejection do not cure the deficiencies of the primary references.

The Qiagen method is irrelevant as the subject invention is focused on a pre-treatment for blood that can be combined with existing blood sample preparation methods like the Qiagen product. Hallick *et al.* and Von Pape *et al.* teach chemistries that are different than what is claimed in the subject invention. Hallick *et al.* teach that ATA is a general nuclease inhibitor. In fact, ATA is known to inhibit both RNases and the DNase used in the subject invention. The subject invention teaches against the art by using ATA during a nuclease reaction where the purpose is to break down blood cell DNA. The addition of ATA, widely known in the art to inhibit RNases and DNases, to a DNase reaction teaches against the art.

Von Pape *et al.* teach the use of sodium citrate as an anticoagulant. Sodium citrate is used in the proteinase K digestion reagent as a true buffer and not in the reaction chemistry of the streptokinase, plasminogen, phospholipase A_2, and DNase of the claimed invention with blood.

Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, 16, 23, and 24 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), and Diez *et al.* (1999), and further in view of Sanyal *et al.* (1997). The Zhang *et al.*, Cassels *et al.*, Smith *et al.*, and Diez *et al.* references are cited for their teachings as before. The Examiner asserts that the Sanyal *et al.* (1997) reference teaches that an ordinarily skilled artisan can perform PCR from RNA by removing contaminating DNA by adding DNAse I in sample preparation, followed by inactivating the DNAse I by heat treating the sample in the presence of EDTA. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.*, Cassels *et al.*, Smith *et al.*, and Diez *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, 16, 23, and 25 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), and Hughes *et al.* (2001) and further in view of Diez *et al.* (1999). The Zhang *et al.*, Cassels *et al.*, Smith *et al.*, and Diez *et al.* references are cited for their teachings as before. The Examiner asserts that the Hughes *et al.* (2001) reference teaches that an effective way to purify high quality RNA is to add RNAse inhibitor DEPC to a sample before PCR is performed. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.*, Cassels *et al.*, Smith *et al.*, and Diez *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, 16, and 27-30 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), and Semple *et al.* (2000). The Zhang *et al.*, Cassels *et al.*, and Smith *et al.* references are cited for their teachings as before. The Examiner asserts that the Semple *et al.* (2000) reference teaches that the protease inhibitor ecotin is a powerful anticoagulant produced by some varieties of *E. coli*, that ecotin inhibits portions of the protease cascade that leads to fibrin clot formation and suggest that ecotin's activity could be useful in various applications in preventing thrombosis. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.*, Cassels *et al.*, and Smith *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, 16, 31, and 32 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), and Wang *et al.* (2001), and Wang *et al.* (2000). The Zhang *et al.*, Cassels *et al.*, and Smith *et al.* references are cited for their teachings as

before. The Examiner asserts that the Wang *et al.* (2000) reference teaches that an endogenous cannabinoid is often upregulated by macrophages in the course of septic shock, and thus is a method of identifying those patients in septic shock, and that HPLC is an effective way to measure the cannabinoids in serum. The Examiner asserts that Wang *et al.* (2001) further teach that a surface plasmon resonance biosensor also allows for detection of the cannabinoid. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.*, Cassels *et al.*, and Smith *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, 16, and 33 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), and Hallick *et al.* (1977). The Zhang *et al.*, Cassels *et al.*, Smith *et al.*, and Hallick *et al.* references are cited for their teachings as before. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.*, Cassels *et al.*, Smith *et al.*, and Hallick *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, 16, 33, and 34 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), Hallick *et al.* (1977), and Lee *et al.* (1998). The Zhang *et al.*, Cassels *et al.*, Smith *et al.*, and Hallick *et al.* references are cited for their teachings as before. The Examiner asserts that the Lee *et al.* (1998) reference teaches that Triton X-100 can be added to clinical serum specimens in a process of isolating DNA that is to be used for PCR identification of a pathogenic bacteria. Applicants respectfully traverse these grounds of

rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.*, Cassels *et al.*, Smith *et al.*, and Hallick *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, 16, 33, and 35-38 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), Hallick *et al.* (1977), Zhang *et al.* (1999), and Pierre *et al.* (1995). The Zhang *et al.*, Cassels *et al.*, Smith *et al.*, and Hallick *et al.* references are cited for their teachings as before. The Examiner asserts that the Zhang *et al.* (1999) reference teaches that steroidal saponins have properties of inhibiting platelet aggregation and have an effect on hemolysis and thus could have use in antithrombotic applications. The Examiner asserts that the Pierre *et al.* (1995) reference teaches that HECAMEG is a useful detergent to add when solubilizing membranes and that HECAMEG has been used specifically to isolate mycoplasma bacterial surface antigens. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.*, Cassels *et al.*, Smith *et al.*, and Hallick *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references.

The methods of the present invention can destroy the nuclear membrane and the blood cell DNA contained inside without disrupting bacterial cell walls. Disruption of bacterial cell walls would expose the bacterial DNA to DNase.

Applicants respectfully assert that addition of enough detergent to lyse nuclear membranes will also lyse delicate clinical pathogens such as *Pseudomonas aeruginosa*. Those pathogens are made undetectable by PCR under such detergent conditions when DNase is present during the detergent incubation period.

Saponin and HECAMEG are used in one embodiment of the claimed method as detergents to drive the reaction of phospholipase A_2 with the endoplasmic reticulum which comprises the cell nucleus membrane. The cell nucleus will not efficiently break down in a reasonable time period (1 – 2 minutes to support rapid diagnostics) with only phospholipase A_2, or only saponin or only HECAMEG at the levels described.

The use of Saponin and HECAMEG with phospholipase A_2 is not taught or suggested by any of the cited references. There is no biochemical reason in the art to combine these specific detergents with an enzyme used to test for endogenous components in clinical samples or research on micelle structure. It is not obvious to use Saponin and HECAMEG with phospholipase A_2. In fact, Phospholipase A_2 activity is known in the art to be inhibited by a wide range of detergents. The subject invention teaches against the art by using these detergents with phospholipase A_2. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 1, 2, 11, 16, 33, and 35-43 are rejected under 35 USC §103(a) as obvious over Zhang *et al.* (1995) in view of Cassels *et al.* (1987), Smith *et al.* (1982), Hallick *et al.* (1977), Zhang *et al.* (1999), Lee *et al.* (1998), and Pierre *et al.* (1995). The Zhang *et al.* (1995), Cassels *et al.*, Smith *et al.*, Hallick *et al.*, Zhang *et al.* (1999), Lee *et al.*, and Pierre *et al.* references are cited for their teachings as before. Applicants respectfully traverse these grounds of rejection.

Applicants respectfully assert that the cited references, whether taken alone or in combination, do not teach or suggest Applicants' claimed invention. Applicants respectfully reassert and hereby incorporate by reference on their entirety the remarks submitted herein in regard to the teachings of the Zhang *et al.* (1995), Cassels *et al.*, Smith *et al.*, Hallick *et al.*, Zhang *et al.* (1999), Lee *et al.*, and Pierre *et al.* references. The secondary references cited under this rejection do not cure the deficiencies of the primary references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

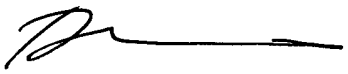
It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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DRP/kmm
Attachments: Petition and Fee for Extension of Time Under 37 CFR §1.136(a)
Amendment Transmittal Letter